



Bedtime Administration of Melatonin to Healthy Females Does Not Alter Menstrual Characteristics

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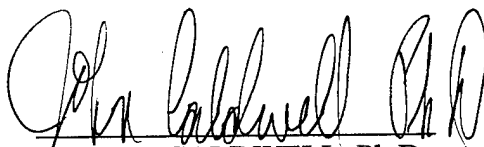


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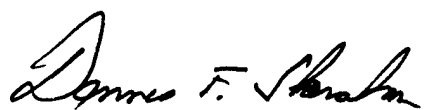
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Introduction

Melatonin, a natural hormone which has been shown to resynchronize circadian rhythms and induce sleep in humans (Arendt et al., 1987; Dawson and Encel, 1993; Reiter, 1991; Wurtman, 1986), is currently being marketed widely as a dietary supplement to alleviate desynchronosis (desynchronization of physiological and behavioral rhythms) and assist in obtaining quality sleep. Desynchronosis often results from rapid shifts in work schedules from day to night, or from shifts in the light-dark cycle due to time zone crossing. Symptoms resulting from desynchronosis include fatigue, sleepiness, lethargy, insomnia, gastrointestinal tract disorders, and poor mental performance (for review see Comperatore and Krueger, 1990). Melatonin therapy has been demonstrated in several studies to be effective in preventing sleep loss and in maintaining alertness following travel across multiple time zones (Arendt and Broadway, 1987; Comperatore et al., 1996a; Petrie et al., 1989). Thus, melatonin can be a potentially effective chronobiotic and ameliorate desynchronosis during travel.

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenously occurring hormone produced by the pineal gland in the absence of bright light. In humans, melatonin synthesis reaches peak levels during the night and lowest levels during the day. Known side effects of melatonin chronobiotic doses (5-10 mg) are limited to sleepiness, fatigue, and reduced alertness shortly after administration, but not upon awakening (Arendt et al., 1987; Comperatore et al., 1996a; Petrie et al., 1989). However, in females, due to a potential inhibitory influence of melatonin over the hypothalamo-pituitary-ovarian axis (Aleem, Weitzman, and Weinberg, 1984; Nordlund and Lerner, 1977), melatonin use may be associated with secondary disruptions of the menstrual cycle. Although the exact relationship between melatonin and the monthly cycle in females is unclear, there is considerable evidence for interaction between melatonin and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Cagnacci, Elliott and Yen, 1991; Diaz et al., 1993; Nordlund and Lerner, 1977; Penny, Stanczyk, and Goebelsmann, 1987; Voordouw et al., 1992). Can melatonin be used safely by females to reduce desynchronosis resulting from travel, or could short-term use of this nonprescription hormone result in disruption of the menstrual cycle? Such disruption could at the very least increase the already stressful conditions associated with deployment.

The study reported here was designed to investigate the effect of exogenous melatonin (10 mg) on menstrual characteristics in normally cycling healthy females when given at bedtime (2300 hours) for 7 consecutive nights during the late follicular and early luteal phase of the monthly cycle. We also questioned whether cognitive performance upon awakening and throughout the day might be altered by this melatonin regimen; however, those results will be discussed in a separate report.

Methods

Subjects

Subjects were 17 healthy females between the ages of 18 and 36 (mean=27.2), nonpregnant, and not taking any medication that could interfere with normal ovulatory events. Additional requirements were menstrual cycles between 21-36 days with no variation more than 3 days above or below the previous cycle for each volunteer, and no oral contraceptive use within 3 months prior to participation. The study was double blind and placebo controlled, and all participants were assigned randomly and exhaustively to either a melatonin (n=8) or placebo (n=9) group. The 8 melatonin volunteers had a mean age of 26.5, while the mean age of the placebo group was 27.8. All participants gave their informed consent and were advised of their right to withdraw at any time. Participants were asked to refrain from consuming alcohol, caffeinated beverages, or any type of medication with known central nervous system effects during the in-house portion (7 days) of their 3 months of participation. Use of nonsteroidal anti-inflammatory drugs such as ibuprofen were limited also since they have been found to decrease melatonin synthesis (Badia, Myers, and Murphy, 1992).

Melatonin and dose administration

Melatonin was obtained from Regis Chemical Company, St. Louis, Missouri, and encapsulated by Regional Services, Inc., Boston, Massachusetts. The 10-mg dose used in this study was selected because of its lack of toxicity (Sugden, 1983), short half-life, lack of side effects, sleep induction effects, and its already demonstrated efficacy in maintaining sleep and alertness in males traveling across multiple time zones (Comperatore et al., 1996). In order to avoid confounding the study with unwanted changes in sleep onset induced by melatonin's chronobiotic properties, the time of day selected for melatonin or placebo administration (2300 hours) fell in the less sensitive zone of the phase response curve for melatonin (Lewy et al., 1995).

Procedure

The first and third months of participation involved collection of information on the timing of menses and ovulation, menstrual regularity, and LH and FSH levels. On each day of both months, subjects were asked to provide a sample of first void urine. Menstrual regularity data (questionnaire) were used to document the timing of menses and to approximate the scheduling of the 7 days comprising the preovulatory LH surge for the in-house portion of menstrual cycle 2. The timing of menses and the preovulatory LH surge was used to document any advances or delays of the menstrual cycle related to the melatonin administration regimen. Since this information could not be obtained from pregnant volunteers, pregnancy tests were conducted throughout the study beginning on day 1 of cycle 1.

During cycle 2, subjects reported to the sleep laboratory at the U.S. Army Aeromedical Research Laboratory (USAARL), Fort Rucker, Alabama, at 0630 on day 10 and lived in the laboratory until 0800 on day 17 when they were released after a brief medical evaluation. Melatonin (10 mg) or placebo was given at 2300 on days 10 through 16. In addition to first void urine samples, subjects were asked to provide specimens every 2-3 hours from 0630 until 2300. Subjects also were asked to drink at least an 8-oz glass of water between voids, but they were not encouraged to consume liquids in excess. Vital signs were recorded at bedtime and upon awakening during dose days.

Cognitive data were collected throughout days 11-16 (cycle 2) and used to determine the time course of the effects of the 10-mg melatonin dose on cognitive function. Those results will be presented in a separate report (see Comperatore et al., 1996b).

Biochemical assays

LH and FSH assays

Hormone levels in urine were used to identify the monthly surge in LH, as well as to determine whether pharmacological levels of melatonin inhibit LH and/or FSH release. Urine samples, collected daily at rise time during the three complete menstrual cycles of the study period, and those collected every 3 hours while awake and just prior to dose during drug administration days, were used. Urinary levels of LH and FSH were measured by direct immunoassay using the Abbott IMx* automated benchtop immunochemistry analyzer system (Fiore et al., 1988). All urine samples were stored at 1-4°C in the USAARL biochemistry lab and assayed the same day or frozen until analysis.

Pregnancy testing

Tests for human chorionic gonadotropin (hCG) were used to detect pregnancy. For maximum sensitivity at critical times during the study (the first day of cycle 1, the first day of melatonin/placebo administration), pregnancy tests were done on samples of blood. Serum levels of hCG were measured by direct immunoassay using the Abbott IMx* automated benchtop immunochemistry analyzer system, which has a sensitivity of about 2 mIU/ml for hCG in serum.

Melatonin assay

Urinary levels of 6-sulphatoxymelatonin (aMT6s) were measured by direct radioimmunoassay (RIA) from specimens collected during days 10-17 of menstrual cycle 2. Urine samples were refrigerated and frozen if not assayed within a day. RIA kits for aMT6s were obtained from American Laboratory Products Company* and from Stockgrand Ltd*.

* See manufactures' list at Appendix

Data analysis

The LH surge was identified for each menstrual cycle (1-3). For each subject, the day(s) of the LH surge for each month of participation served as a dependent variable. These data, as well as LH assay data obtained throughout days 11-16, were analyzed using a mixed factorial analysis of variance with two levels of the between subjects factor Drug (melatonin and placebo) and six levels of the within subjects factor Day (cycle days 11-16). A similar analysis strategy was used for FSH levels. The aMT6s assays on urine samples collected during days 11-16 of menstrual cycle 2 were used to document elevated levels induced by the administration of the 10-mg dose of melatonin.

For each subject, menses onset day and duration was recorded for the three consecutive menstrual cycles. The timing of menses was measured from menstrual cycle numbers 1 to 2 and from 2 to 3. The periodicity established by these observations constituted two additional dependent variables capable of reflecting changes in menstrual regularity. Menses periodicity data which occur outside of the expected range of menstrual variability (± 3 days), indicated by participants in the volunteer screening questionnaire, were considered significant. In this case, strict use of statistical analysis would ignore idiosyncratic characteristics of menstrual regularity.

Results

Melatonin metabolite concentrations

Levels of aMT6s were assayed from urine samples provided while volunteers were in-house during cycle 2. Although there was considerable variation between volunteers, there was no question as to the identity of those in the melatonin group. The mean level of aMT6s in the first void of all 17 volunteers on day 10 of cycle 2 (prior to first dose) was 26.8 ± 12.8 ng/ml. The highest value recorded for any placebo volunteer during their in-house stay was 47.8 ng/ml. The mean level of aMT6s in the first void of the eight melatonin volunteers on day 11, following their first administration of melatonin, was 20622.4 ± 9339.8 ng/ml. Figure 1 shows the mean aMT6s concentration for each of the seven daily samples for one member of the placebo group during the 6 in-house test days (11-16), as well as the same information for one member of the melatonin group. Note in Figure 1 that not only are aMT6s levels tremendously elevated in the first void from the melatonin volunteer, but they remain elevated throughout the day. Although the elevation in aMT6s in the first void sample is consistent in all melatonin volunteers, the sustained elevation above placebo levels throughout the day is not. Evidence of individual variation is provided by levels of aMT6s returning to placebo level late in the day for some volunteers, but not for others. The first two samples in the morning are the highest in both the melatonin and placebo groups, and after a midday fall, volunteers in both groups often show an increase in aMT6s in the sample collected at bedtime (see Figure 1).

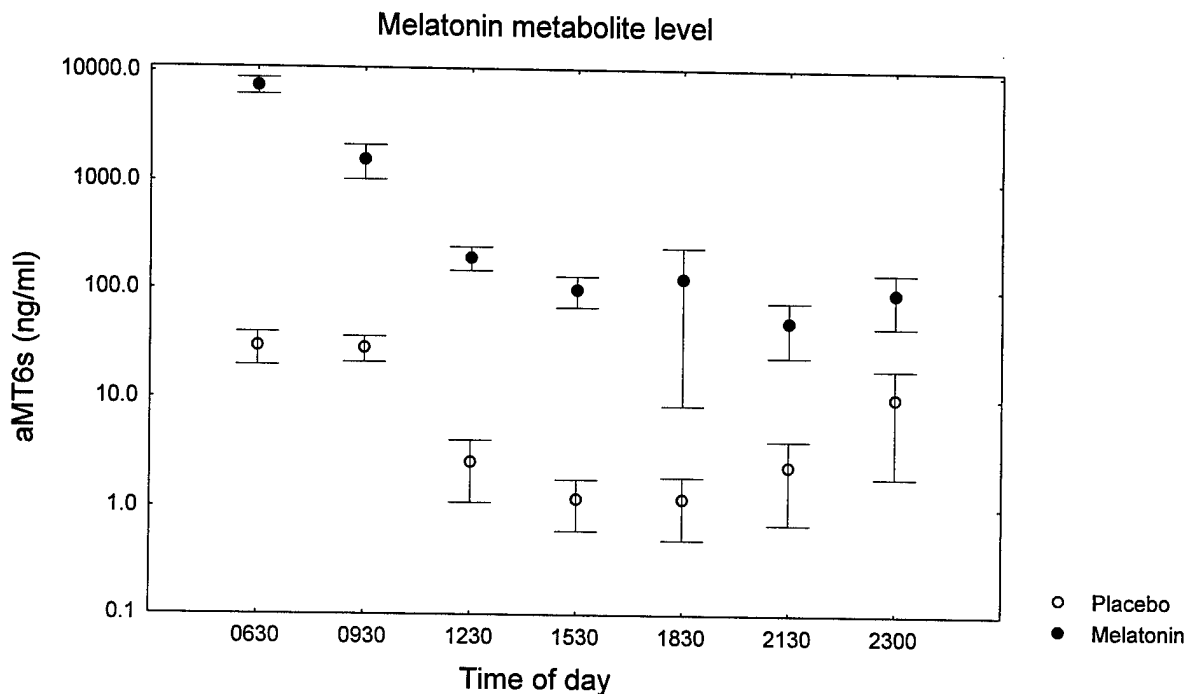


Figure 1. Melatonin metabolite level in urine. Mean aMT6s concentration for each of the seven daily samples during the 6 in-house post-administration days for one member of the placebo group and one member of the melatonin group. Error bars indicate \pm the standard error of the mean.

Awareness of symptoms following melatonin

Following the in-house stay during cycle 2, all volunteers were asked if they thought they had received melatonin. Three of the 17 responded "yes," but none of the three were in the melatonin group. Individuals from both the melatonin and placebo group reported sleeping well at night, and having difficulty awakening in the morning. Vital signs were checked upon awakening and before getting out of bed in the morning, and they were never outside of the normal physiological range. One member of the melatonin group felt nauseous on two separate mornings, but did not feel she was taking melatonin. There apparently are no consistent symptoms the next day following 10 mg of melatonin taken at bedtime. It is also quite apparent from our observations that volunteers are unable to distinguish between melatonin and placebo.

Length of menses and menstrual cycle after melatonin

Although our sample size was limited ($n=8$ for melatonin), there were no consistent changes in either length of menstrual cycle or menses. Since most volunteers indicated that an occasional increase or decrease in the length of their menstrual cycle of up to 3 days was within normal limits, we elected to follow the same guidelines. Therefore, a change of more than 3 days

had to occur before it was considered physiologically significant. Table 1 lists the menstrual cycle length based upon the first day of menses (CL-A, 3 cycles), the cycle length based upon the LH surge for cycle 1-2 and 2-3 (CL-B, 2 cycles), and the length of menses (ML, 3 cycles), for each of the 17 participants.

TABLE 1: MENSTRUAL CYCLE CHARACTERISTICS

Vol #	Drug	CYCLE 1			CYCLE 2		CYCLE 3		
		CL - A	CL - B	ML	CL - A	ML	CL - A	CL - B	ML
01	M	29	27	4	28	4	25	30	3
02	M	26	26	6	26	5	25	25	6
03	P	28	25	3	29	3	29	31	4
04	P	29	27	5	27	5	32	33	5
05	M	24	24	5	27	5	24	26	4
06	P	30	28	5	26	5	32	30	5
07	P	31	35	4	26	4	24	16	3
09	M	26	28	3	28	4	25	27	5
11	M	26	28	6	28	6	32	32	4
12	M	30	28	6	27	5	31	32	5
15	M	27	27	8	27	7	28	28	7
16	P	25	25	5	27	5	27	27	5
17	P	27	29	5	29	3	29	28	5
19	P	27	26	5	22	4	27	24	6
20	P	26	26	8	24	5	21	24	4
24	P	34	35	6	32	6	31	30	6
28	M	31	27	5	26	6	25	25	7

CL-A Cycle length based upon first day of menses

CL-B Cycle length based upon LH peak

ML Length of menses

Remembering that participants received melatonin starting on day 10 of cycle 2, based upon menses onset, the length of cycle 2 compared to cycle 1 was shorter for three and the same for six placebo subjects (overall mean 2.9 day difference), and shorter for one, and the same for seven melatonin subjects (overall mean 2.0 day difference). Comparing cycle 3 to cycle 2, overall length was greater for three and the same for six in the placebo group (overall mean difference=2.4 days), and greater for two and the same for six in the melatonin group (overall mean difference=2.5 days). Figure 2 shows this information plotted as change in cycle length for each subject; it is readily apparent that there is no systematic change for either placebo or melatonin group. The horizontal dotted lines in the figure indicate the minimum change which must be exceeded for physiological significance.

Figure 3 shows a comparison between cycle lengths as determined by the number of days between LH peaks. The number of days between the identified LH surge is compared between cycles 1 to 2 and 2 to 3. Again using 3 days as a minimum for physiological significance (dotted lines), the melatonin group had two increases and six unchanged, and the placebo group had two increases, two decreases, and five unchanged. As was the case with changes in cycle length determined by first day of menses, there was no systematic change.

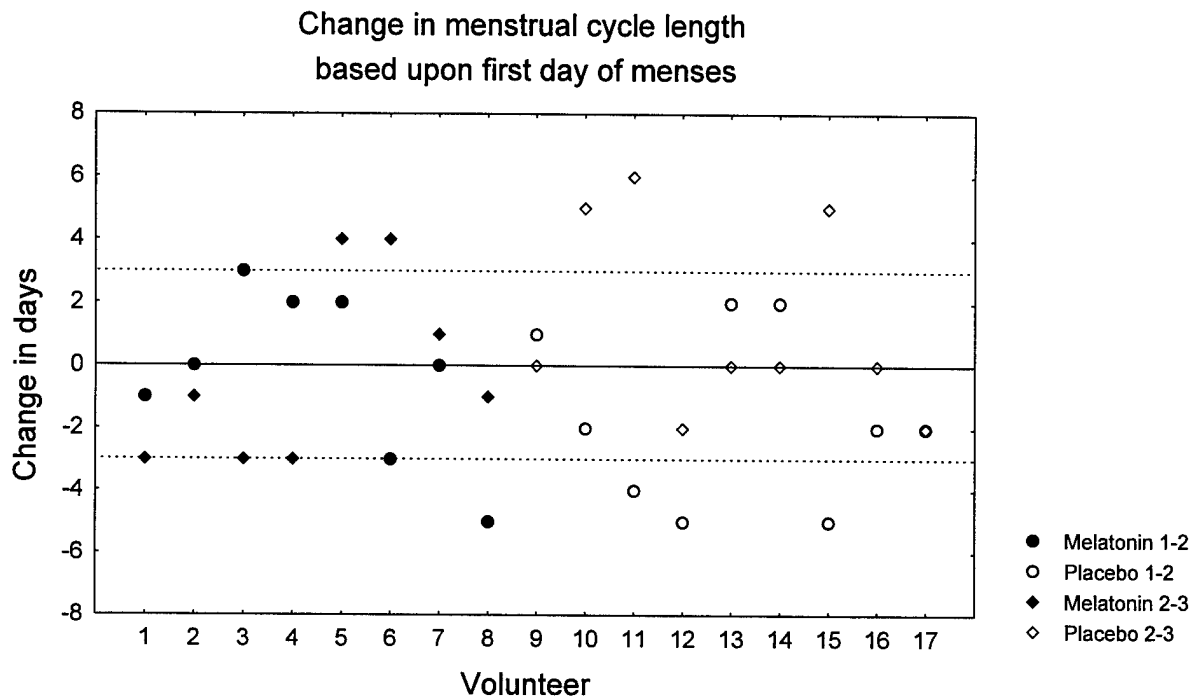


Figure 2. Change in menstrual cycle length based upon first day of menses for all members of both the melatonin and placebo groups between cycle 1 and 2 and between cycle 2 and 3. The dotted lines indicate 3 days which we have selected to indicate physiological significance.

Since the in-house dose days occurred after menses in cycle 2, both cycle 1 and cycle 2 serve as controls for menses. Average length of menses for the 3 cycles was 5.4, 5.3, and 5.1 days for the eight melatonin group members, and 5.1, 4.4, and 4.8 for the nine members of the placebo group. The average length of menses for the first 2 cycles taken together was 5.31 for the melatonin group and 4.78 for the placebo group. For cycle 3, the only post-melatonin month, the average length of menses was 5.13 days for the melatonin group and 4.78 days for the placebo group. Figure 4 shows changes in menses between cycles 1 and 2 (2 pre-melatonin cycles) and between cycles 2 and 3 (a pre- and a post-melatonin cycle) for all members of both melatonin and placebo groups. No members of either the placebo or melatonin groups demonstrated changes of greater than 2 days in the post-melatonin cycle, and only two placebo volunteers and one melatonin volunteer showed a 2 day change. Inspection of Figure 4 shows no apparent difference in melatonin or placebo groups between the pre- and post-melatonin cycles. A two way analysis of variance applied to the Drug (melatonin and placebo) and the Cycle (pre-

and post-melatonin) factors revealed that the melatonin regimen did not significantly change the duration of menses ($F[1,15] = 1.869$; $p = 0.192$).

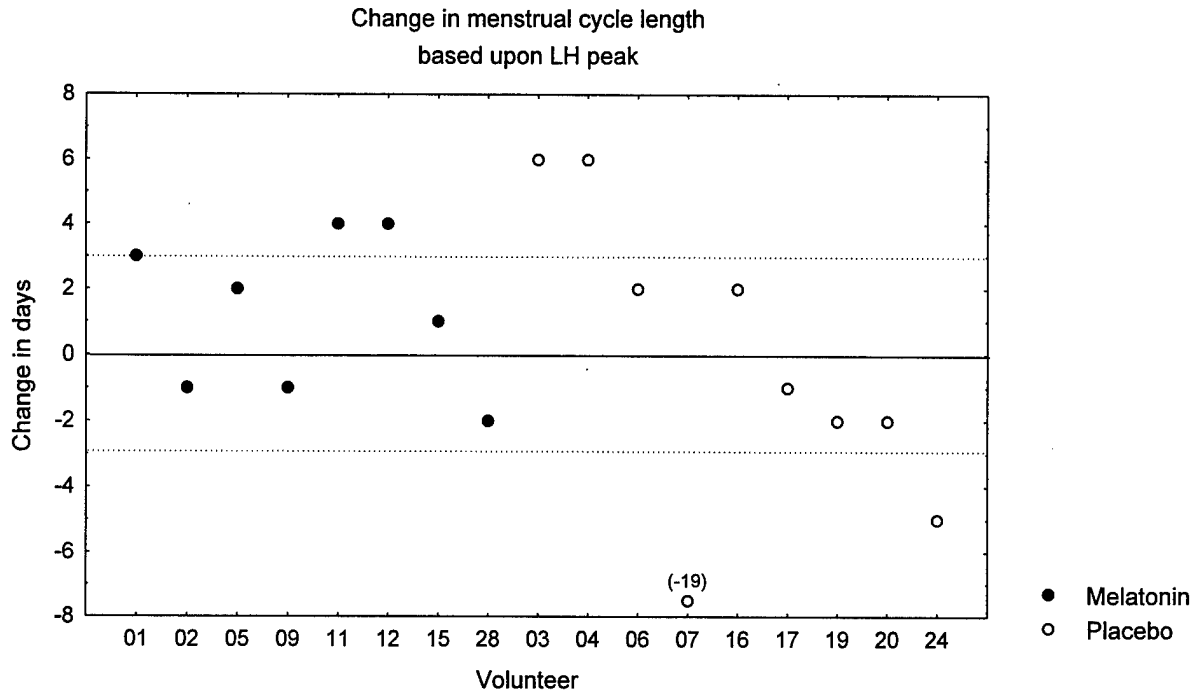


Figure 3. Change in menstrual cycle length based upon timing of LH peak for members of both the melatonin and placebo groups between cycles 1-2 and 2-3. The dotted lines indicate ± 3 days which we have selected to indicate physiological significance.

Use of IMx technology with urine samples

Since there are suggestions in the literature that melatonin may influence levels of LH and FSH, we not only analyzed daily first void urine samples for these hormones, but we also analyzed seven samples daily for each volunteer while in-house. Because the IMx system is recommended for detecting LH and FSH in plasma, we wanted to verify the reliability of the system for LH and FSH activity in urine. Eight volunteers were asked to check their first void samples with the Whitehall-Robbins Clearplan Easy* one-step ovulation predictor, which works by measuring LH in the urine utilizing monoclonal antibody technology. Six of the eight had positive results on the ovulation predictor on the same day that IMx results showed the LH peak. The other two volunteers had positive results on the ovulation predictor within 2 days of the IMx determined LH peak. This is not surprising since LH levels during the mid-cycle surge often remain elevated for 2 days or more. There are no similar indicators for FSH in urine, but since an increase in FSH is expected about the same time as the LH surge, and one was seen in our samples, we assumed the IMx also provided an accurate indication of FSH activity as well.

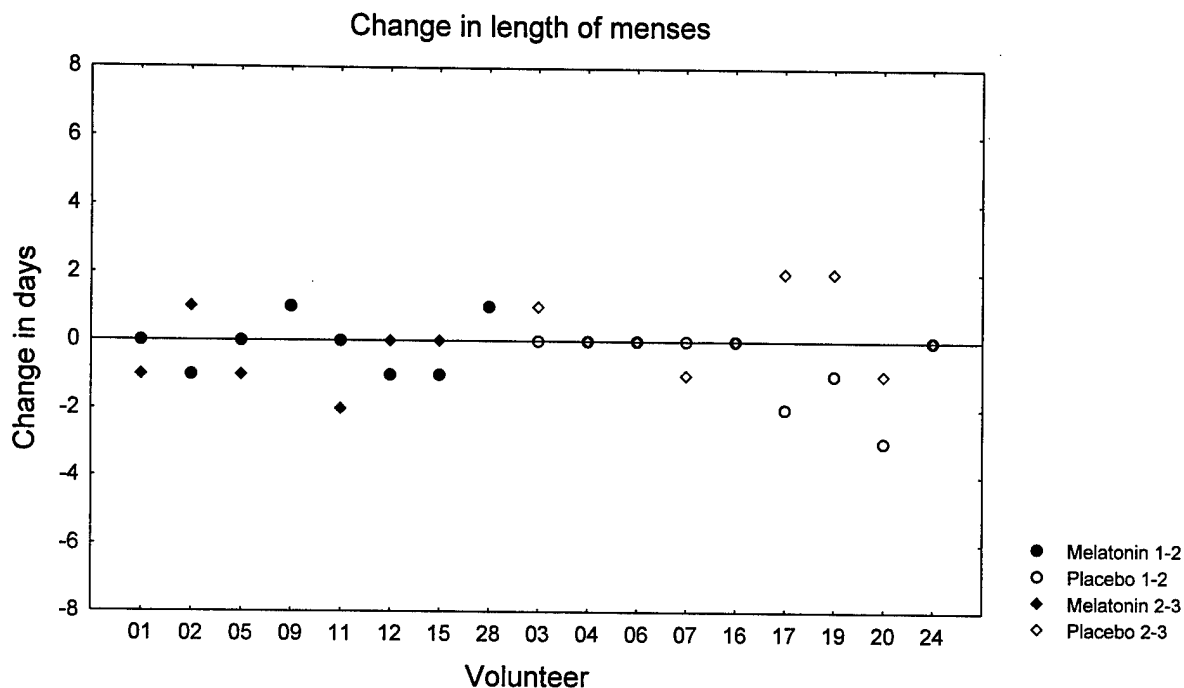


Figure 4. Change in length of menses for all members of both the melatonin and placebo groups between cycles 1 and 2 and between cycles 2 and 3.

Melatonin effects on LH and FSH

Figure 5 is a typical plot of LH and FSH levels against day for the entire three cycles of the study from one of our volunteers. In most cases the baseline activity for LH is quite low and there is a strong elevation during the preovulatory surge. Often the surge persists for 2 or 3 days as in cycle 1 of Figure 5 (2 LH peaks separated by a day). Even in cycle 1 of this figure, which demonstrates the smallest of the three peaks, LH is elevated about six times above baseline. FSH results are usually less clear, with two or more monthly increases, less peak amplitude, and less peak separation from baseline activity. Because of these reasons, we are less comfortable addressing differences in FSH activity. Figure 6 shows LH and FSH levels against day for the entire three cycles of the study from the volunteer showing the strongest LH and FSH peaks. Note the tremendous separation from baseline for both LH and FSH during all three cycles. Note also the scale difference between Figures 5 and 6 (40 mIU vs 100 mIU). Comparison of Figures 5 and 6 provides a good indication of the individual variation seen in these data.

Figure 7 shows mean LH levels across days for both the placebo and melatonin groups for each of the seven sampling times during the in-house stay. Figure 8 provides the same information for FSH. There are no statistically significant differences in either case. It is readily apparent from the figures that the mid-cycle surge for LH and FSH appears during the end of the in-house week. LH/FSH increase above baseline is especially pronounced during days 13-16.

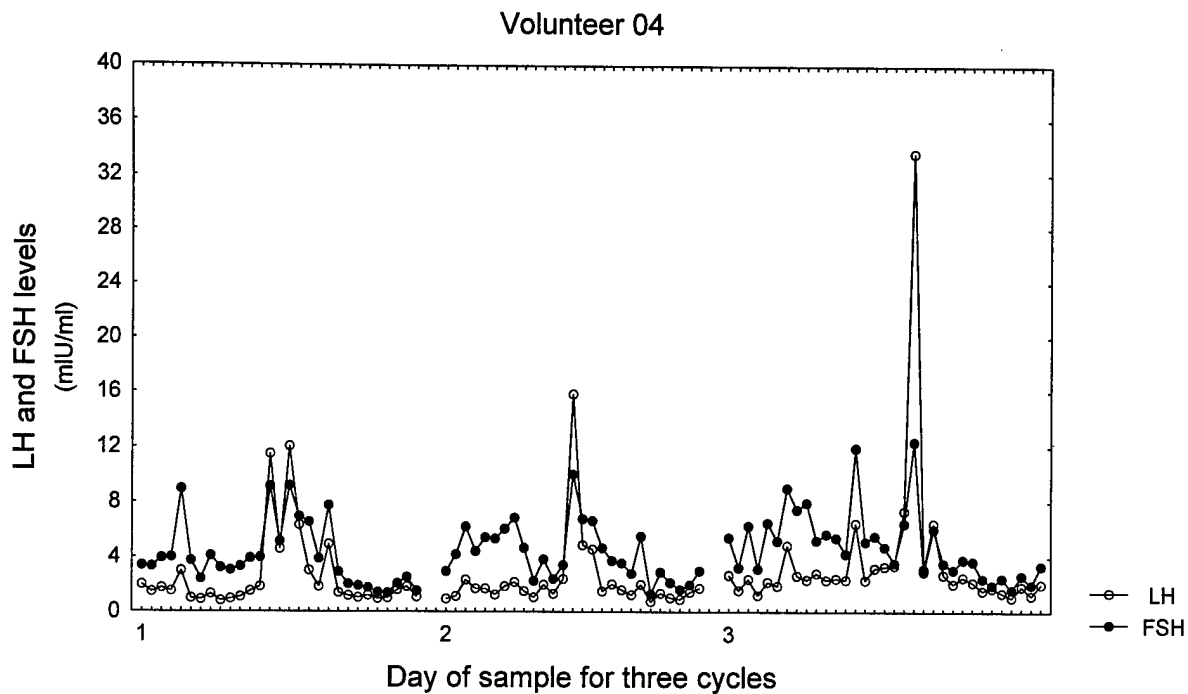


Figure 5. Daily LH and FSH levels over three cycles. LH and FSH levels for each day of the three cycles of participation for one of the placebo volunteers.

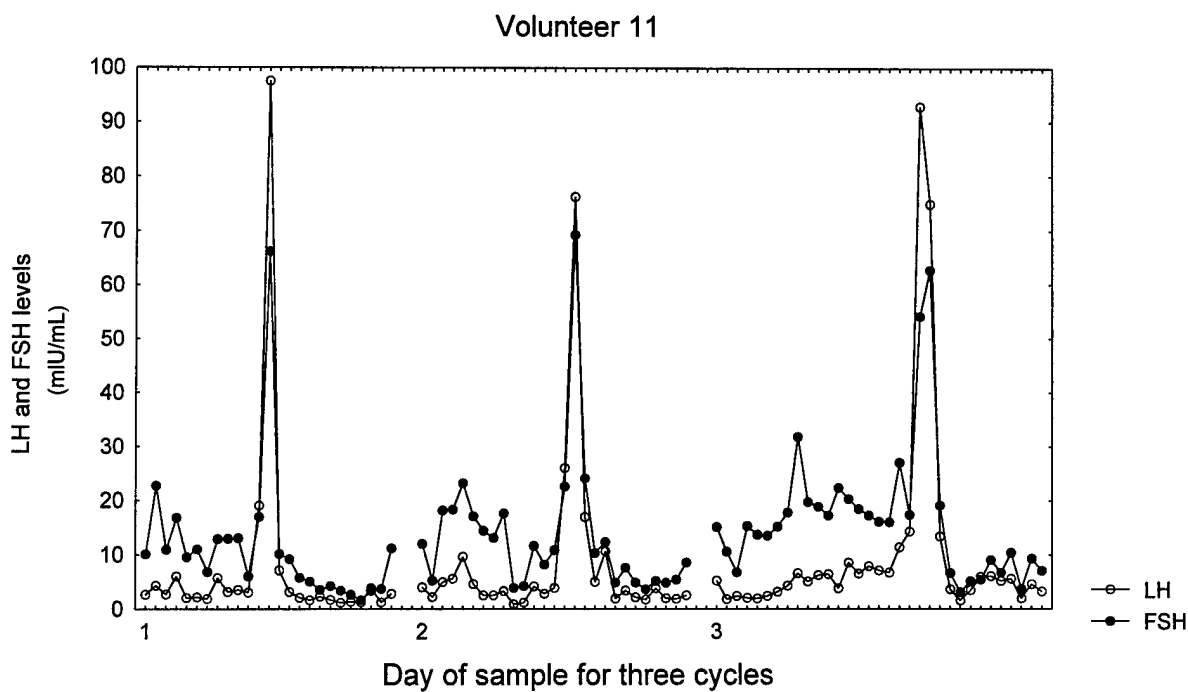


Figure 6. Daily LH and FSH levels over three cycles. LH and FSH levels for each day of the three cycles of participation for the volunteer (melatonin group) demonstrating the strongest LH and FSH peaks.

Although there appears to be a trend for greater elevation in the melatonin group, especially for LH in Figure 7, a three-way analysis of variance applied to the Drug (melatonin and placebo), Day (7 days), and Time of day (7 samples per day) factors did not reveal significant group differences in LH levels ($F[1,6] = 1.0252$; $p = 0.35$) or interactions between the factors ($F[36,216] = 1.011$; $p = 0.458$).

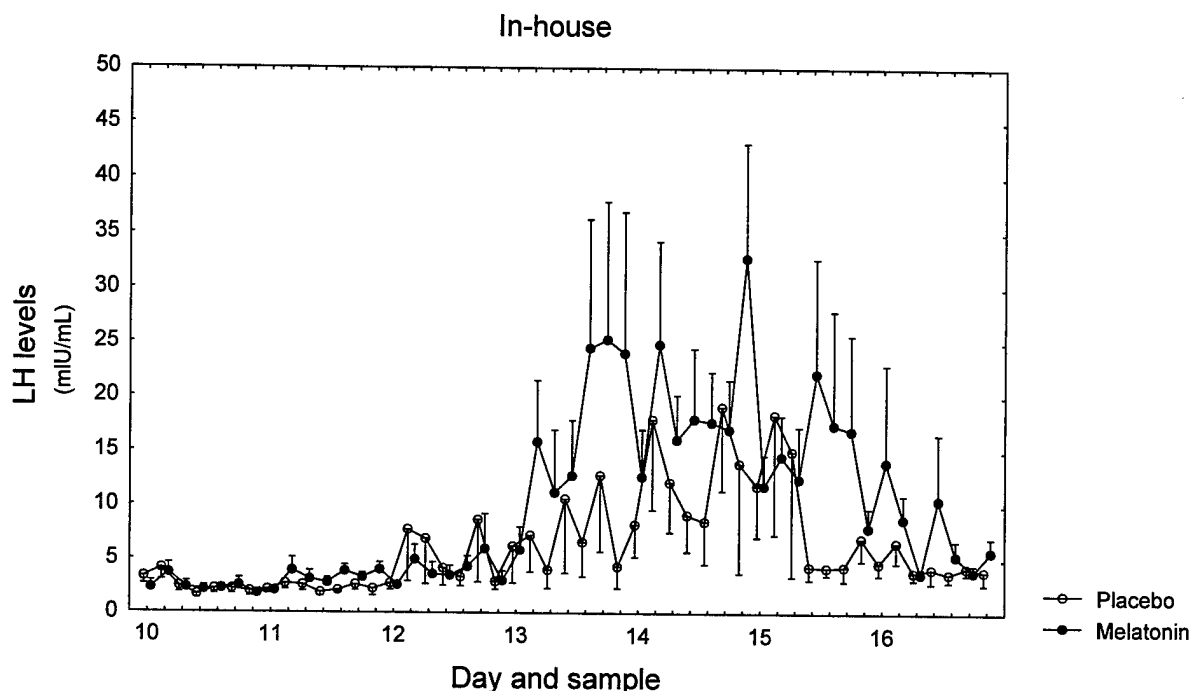


Figure 7. Mean LH levels during the seven day in-house stay for both melatonin and placebo groups.

Our original inspection of the LH data from first void samples suggested decreased levels in the melatonin group during the cycle 2 surge, following their in-house stay and melatonin administration. Closer inspection showed decreased levels for both the melatonin and placebo groups, indicating that the decrease during cycle 2 could not be attributed to melatonin. Figure 9 shows the mean peak LH values for the three cycles for both the melatonin and placebo groups, as well as the mean peak values from multiple daily samples while in-house. Note that the in-house values for both melatonin and placebo groups are identical and higher than any of the values for the other 3 months. Table 2 lists the LH and FSH peaks determined from first void samples for each of the three cycles, as well as the LH and FSH peak determined from 7/day urine samples during the in-house stay. Examination of this table (compare the cycle 2 and the cycle 2 in-house columns) reveals that none of the first void samples during cycle 2 caught the LH peak. It was always in a sample later in the day. Fifteen of our 17 volunteers, providing urine samples every 3 hours from 0630 to 2300, demonstrated their LH surge while in-house. Of those 15, 4 exhibited their peak LH level at 0930 and the other 11 were all in the afternoon. Unfortunately, this means we do not know the actual value of the LH peak for cycles 1 and 3, although it was almost certainly higher than recorded in our first void samples. The fact that the

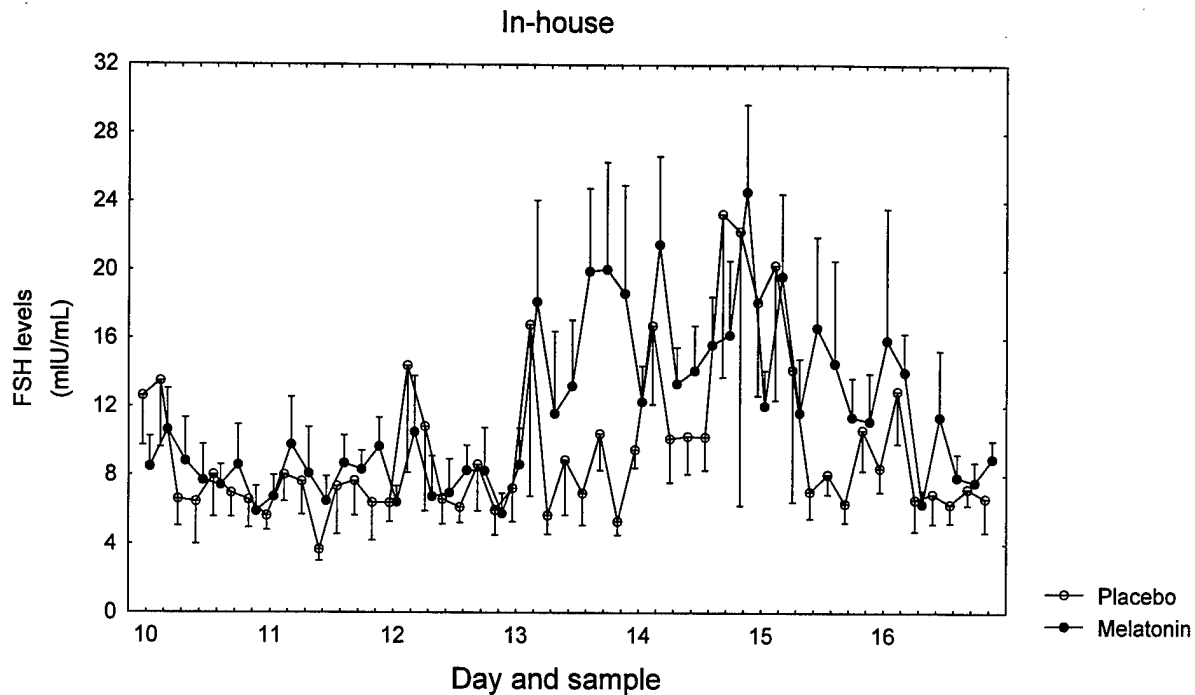


Figure 8. Mean FSH levels during the seven day in-house stay for both melatonin and placebo groups.

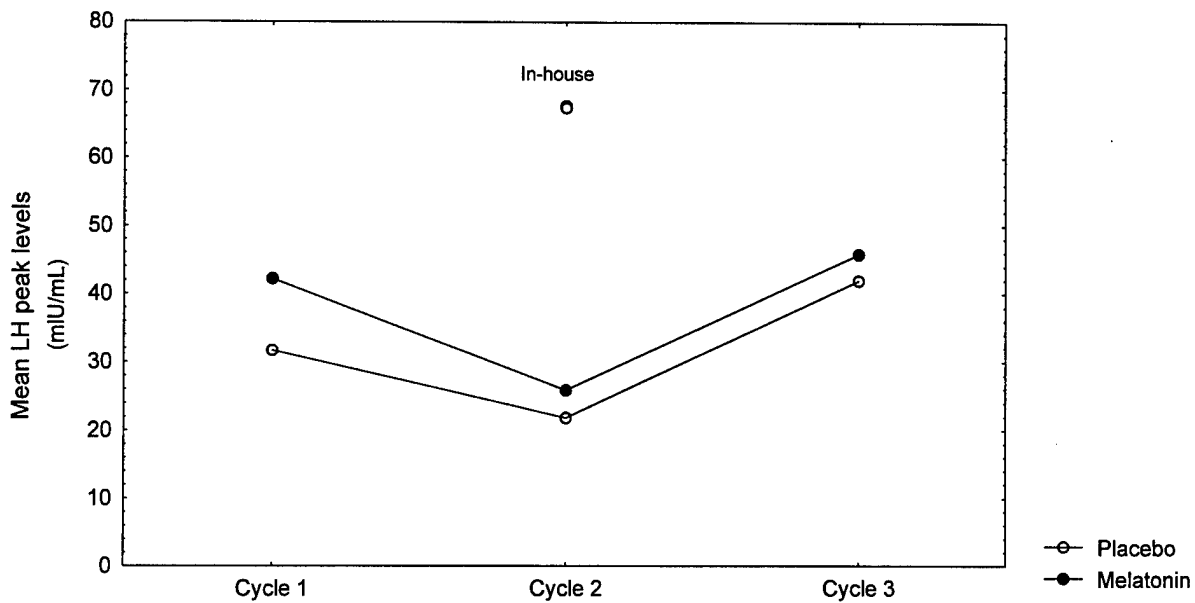


Figure 9. Mean peak LH levels for three cycles of participation. Mean peak LH levels based upon first void urine samples for both the melatonin and placebo groups for each of the three cycles as well as the mean peak value based upon multiple daily samples during the in-house phase of cycle 2.

peak level of the LH surge during cycle 2, based upon multiple daily samples while in-house, was identical for both melatonin and placebo groups suggests that there is no difference between the two groups.

TABLE 2: LH AND FSH PEAK VALUES

Vol #	Dose	Cycle 1		Cycle 2		Cycle2*		Cycle 3	
		LH	FSH	LH	FSH	LH	FSH	LH	FSH
01	M	29.3	39.9	17.1	21.8	46.1	44.6	46.2	46.7
02	M	77.9	99.3	34.1	19.5	108.7	57.9	92.1	68.2
03	P	19.9	27.2	15.4	26.1	NP	NP	7.8	16.9
04	P	12.0	9.2	15.8	10.0	77.1	43.8	33.6	12.4
05	M	18.9	24.3	13.3	14.8	45.4	41.7	5.3	NP
06	P	28.6	17.0	28.7	17.0	67.9	22.9	55.9	27.9
07	P	48.3	25.1	9.4	11.3	18.4	21.6	88.9	24.8
09	M	18.3	34.7	16.3	13.6	85.1	44.0	9.6	21.3
11	M	97.5	66.2	76.3	69.3	93.2	69.3	92.9	62.7
12	M	19.2	14.3	13.6	11.9	41.7	43.0	25.1	23.7
15	M	38.4	17.3	30.0	21.5	85.7	40.9	71.2	32.4
16	P	50.0	35.4	31.3	19.6	63.3	32.4	23.4	18.6
17	P	48.7	50.1	44.3	43.6	104.9	80.0	66.3	46.7
19	P	57.7	150.0	8.8	10.9	45.6	61.8	67.4	150.0
20	P	5.7	19.8	25.0	49.7	94.4	150.0	17.0	21.2
24	P	14.4	8.9	17.8	11.0	NP	NP	17.4	13.8
28	M	37.9	22.4	6.3	8.6	35.6	42.5	24.1	30.7

Cycle 2* Values determined from multiple daily samples while in-house.

Analysis of FSH results were less consistent. Again, based upon analysis of first void samples, six of eight melatonin volunteers showed decreased FSH levels from cycle 1 to cycle 2, while in the placebo group, there were four decreases, two increases, and three with essentially no change. There were no systematic changes in either group with respect to the timing of the monthly surge (see Table 3). For cycle 1 to 2, the melatonin group showed four increases, two decreases, and two with no change, while the placebo group showed four increases, four decreases, and one with no change. For cycle 2 to 3, the melatonin group showed four increases, three decreases, and one with no change, while the placebo group showed four increases, two decreases, and three with no change.

Discussion

We have demonstrated here an apparent lack of effect of melatonin (10 mg), when given at bedtime to normally cycling healthy females during the late follicular and early luteal phase of the monthly cycle, on menstrual cycle length, length of menses, and timing of the LH and FSH monthly rhythms. There were no reported side effects from melatonin administration, and

melatonin volunteers were unable to determine whether they were receiving melatonin or placebo. In addition to hormone levels and menstrual characteristics, cognitive testing was done on the volunteers, both upon awakening in the morning and throughout the day following melatonin administration. Melatonin volunteers performed better than placebo volunteers on some tasks, but not on others. These results will be presented in a separate report (see Comperatore et al., 1996b).

TABLE 3: CYCLE DAY OF LH PEAK

Vol #	Dose Condition	Cycle 1	Cycle 2	Cycle 3
01	M	15	13	15
02	M	13	14	13
03	P	20	18	22
04	P	16	14	20
05	M	15	15	15
06	P	16	14	18
07	P	19	23	11
09	M	13	15	14
11	M	13	16	21
12	M	14	14	18
15	M	13	14	15
16	P	13	13	13
17	P	14	15	15
19	P	14	13	15
20	P	14	15	15
24	P	20	22	19
28	M	19	15	14

During this investigation we determined urinary levels of 6-sulphatoxymelatonin (a major melatonin metabolite found in urine) rather than melatonin itself, because of the difficulties involved with recruiting volunteers willing to submit to frequent blood sampling over 7 days. Following administration of melatonin, melatonin excretion was shown to closely parallel serum melatonin levels for 9 hours (Arendt et al., 1985). After 9 hours, urinary levels tended to be higher than those predicted from serum levels. Urinary levels of aMT6s have been shown to correlate closely, both quantitatively and qualitatively, with plasma melatonin levels in normal humans (Bojkowski and Arendt, 1990; Lee et al., 1995). Based upon the results of our assay for aMT6s, we feel secure in saying that our volunteers in the melatonin group had elevated levels of melatonin upon arising, and probably well into the morning. Whether elevated levels of urinary aMT6s throughout the day provide an accurate indication of melatonin levels in the blood cannot be determined from our data. Published values for a melatonin half life of 60 minutes or less

would argue against elevated blood levels 18-24 hours after melatonin administration. However, changes in cognitive performance throughout the day could suggest sustained elevation in blood levels (see Comperatore et al., 1996b).

Peak serum melatonin concentrations vary by as much as 300 to 10,000 times the normal nighttime levels following melatonin administration (Waldhauser et al., 1984; Vakkuri, Leppaluoto and Kauppila, 1985). Since a major portion of the melatonin in blood is metabolized in the liver and excreted in the urine as the sulfate, it is not surprising to find high levels of aMT6s in the urine following melatonin administration, as reported here. We did not consider body weight (possible metabolic differences) while recruiting our volunteers, or attempt to regulate fluid intake while they were in-house. Differences in metabolism could certainly vary the rate at which aMT6s appears in the urine, and since melatonin was administered at 2300, first void samples could be more or less concentrated from one volunteer to the next depending on fluid intake. This could explain much of the variation in aMT6s levels between volunteers. We saw levels of aMT6s in the melatonin group elevated as much as 1400 times above first void levels in the placebo group. Variation in aMT6s levels between subjects also can arise because of variable gastrointestinal absorption of synthetic melatonin. Absorbed ingested melatonin has been reported to vary by as much as 25 fold among subjects (Waldhauser et al., 1984). Quite often daytime values for placebo volunteers were at or below the detection limit for the assay, while similar values for members of the melatonin group remained elevated (see Figure 1).

Levels of LH and FSH most often are determined through the use of plasma or serum based assays. Since we were interested in determining levels at least daily for 3 months, blood sampling was not a viable option. Urinary LH has been shown to be a reliable marker for plasma LH, since urinary and plasma LH concentrations change in parallel (Kerin et al., 1980). In a study involving the prediction of ovulation required for aspiration of oocytes during spontaneous menstrual cycles, the Abbott IMx* system was shown to be a reliable method for measuring urinary LH and for predicting ovulation (Bischof, Bianchi, and Campana, 1991). More recently, the midcycle serum LH peak was shown to be highly correlated to the measurement of urinary LH metabolites in frozen and thawed first void urine samples (Clough et al., 1992). This was supported in the current study by eight volunteers who used the Whitehall-Robbins Clearplan Easy* ovulation predictor and found good agreement between positive results on the predictor and the IMx results. We therefore felt confident in determining daily LH and FSH levels from urine samples using the Abbott IMx* system.

Based upon IMx determinations of LH and FSH in urine samples from our volunteers, obtained during in-house stays in cycle 2, we found no systematic changes occurring as a result of melatonin administration. While first void samples always missed the absolute peak values of LH and FSH, as revealed by multiple daily samples while in-house, they did not miss the monthly surge.

There is considerable evidence from the animal literature that melatonin administration alters the timing or inhibits the LH surge and inhibits ovulation in female rats (Chiba, Akema and

Toyoda, 1994; Reiter and Sorrentino, 1971; Ying and Greep, 1973). In human females, large doses of melatonin (300 mg and 1 g) have been reported to decrease LH levels (Voordouw et al., 1992; Nordlund and Lerner, 1977). Supporting these findings, young women in athletic training (3-5 year average) have been shown to have higher daytime levels of melatonin and lower levels of LH (Diaz et al., 1993). In contrast, Cagnacci et al. (1991) have reported that melatonin enhances LH during the early follicular phase (days 2-5) without modifying FSH. The effect on FSH generally is reported as minimal or not consistent, in good agreement with our results. As previously discussed, we are unable to address with any certainty the effect of melatonin on absolute levels of LH. We also are unable to predict the effect of melatonin administration at different times of the day or during different times of the monthly cycle. Since it is likely that melatonin would be used earlier in the day during travel across multiple time zones, its specific effect at other times is an important consideration.

The rise in endogenous melatonin in circulation begins after sunset and reaches maximum levels about 0200-0300 (Brown et al., 1985). There is little available melatonin throughout the day. Melatonin receptors are found at many locations both within the central nervous system and in other regions of the body. These receptors, like other classes of receptors, decrease their numbers (down-regulate) with increased availability of melatonin (night) and increase their numbers (up-regulate) with decreased availability of melatonin (daytime) (Gauer et al., 1994; Piketty and Pelletier, 1993; Poon et al., 1994; Tenn and Niles, 1993). Since the nightly rise in endogenous melatonin was well underway at the time of melatonin administration (2300) in this study, fewer receptor sites were available for interaction with exogenous melatonin. Since melatonin receptors should be up-regulated during daylight hours, exogenous melatonin should have its maximum effect on ovulation and menstrual characteristics at that time. We currently are investigating that possibility by administering melatonin at 1300 hours, during the advance region of the melatonin phase response function.

We have demonstrated that in our hands, administration of melatonin (10 mg) at 2300 during the late follicular and early luteal phase of the monthly cycle to normally cycling healthy females has no apparent effect on menstrual characteristics or ovulation. Under these conditions, administration of melatonin (10 mg) appears to be without significant risk.

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Appendix

List of manufacturers

Abbott Diagnostics
A Division of Abbott Laboratories
One Abbott Park Road
Abbott Park, IL 60064

American Laboratory Products Company
P.O. Box 451
Windham, NH 03087

Stockgrand Ltd.
School of Biological Sciences
University of Surrey
Guilford, Surrey
GU2 5XH, UK

Whitehall-Robbins Healthcare
American Home Products Corporation
Five Giralda Farms
Madison, NJ 07940